UNIVERSITY OF CALIFORNIA
COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
BERKELEY, CALIFORNIA

FERTILITY IN SHEEP AS AFFECTED BY NUTRITION DURING THE BREEDING SEASON AND PREGNANCY

R. F. MILLER, G. H. HART, and H. H. COLE

BULLETIN 672
October, 1942

UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

CONTENTS

FERTILITY IN SHEEP AS AFFECTED BY NUTRITION DURING THE BREEDING SEASON AND PREGNANCY[1,2]

R. F. MILLER,[3] G. H. HART,[4] and H. H. COLE[5]

INTRODUCTION

THE PERCENTAGE lamb crop is important to the financial success of the sheep grower. The reproductive capacity of ewes depends upon the successful completion of several processes: ovulation or the liberation of the female germ cell; fertilization of the germ cell; implantation, or the attachment of the embryo to the uterus of the mother, and maintenance of this attachment throughout pregnancy; normal delivery or parturition; and, finally, fostering of the lambs during the nursing period. The normality of each of these reproductive processes depends upon (1) hereditary influences, (2) nutrition, and (3) other environmental factors such as temperature, rainfall, altitude, and sunlight.

This paper concerns the effect of nutrition during the breeding season and pregnancy upon fertility. Since the new data reported here are results of a series of long-time experiments, it was deemed expedient to review certain of the early tests which have already been published in a technical journal.

In California the breeding period is the time when flocks are too frequently maintained under poor feed conditions. This study does not include lactation because in most cases the ewes were not continued on the deficient diets after the close of the breeding season.

First, however, let us briefly consider each of the reproductive processes involved in determining fertility, citing experimental work done elsewhere. This preliminary discussion will serve to acquaint the stockman with the fundamental principles involved in reproduction.

[1] Received for publication February 2, 1942.

[2] The work reported herein was part of an investigation of the relation of nutrition to reproduction which is being carried on in coöperation with the Bureau of Animal Industry, United States Department of Agriculture. It was further financially supported under a Bankhead-Jones project of the same Bureau during the last three years.

[3] Professor of Animal Husbandry and Animal Husbandman in the Experiment Station.

[4] Professor of Animal Husbandry and Animal Husbandman in the Experiment Station.

[5] Associate Professor of Animal Husbandry and Associate Animal Husbandman in the Experiment Station.

THE REPRODUCTIVE PROCESSES

Ovulation and Fertilization.—These may logically be discussed together, being both associated with estrus, or heat. Ewes of certain breeds such as the Dorset and the Australian Merino are believed to come into estrus at 17-day intervals throughout the year, whereas most mutton breeds have a restricted sexual season extending, approximately, from September to February in the northern hemisphere (*23*).[6] According to Marshall (*24*), when Southdown ewes are transferred from the northern to the southern hemisphere, the sexual season is apparently reversed in relation to the calendar year, to conform to the southern hemisphere seasonal cycle. The reversal, furthermore, may occur so quickly that the ewe may have two sexual seasons the year the transfer is made. In such instances, exteroceptive or environmental factors are shown to influence reproduction. Such evidence is pertinent to difficulties involved in breeding when young ewes are moved much shorter distances—for example, from Oregon to California. Others have studied the effect of meteorological or environmental factors upon reproduction, but most of these studies involve changes in nutrition as well. Nichols (*38*) found, for example, some evidence that certain breeds differed from others in their reaction to temperature. According to White and Roberts (*45*), Welsh mountain sheep were more prolific when kept in the lowlands throughout the year than when kept during summer on uncultivated land of such poor quality and high elevation that no ewe lambs and few ewes were wintered there. At castration the number of lambs per hundred ewes was 126 for the former, 86 for the latter. The difference was due to three factors: (1) the number of ewes conceiving, (2) the percentage of twins, and (3) the percentage of still births and deaths.

The term "estrous cycle" refers to a series of changes occurring in the reproductive organs between one period of estrus and the succeeding one. In the ewe the estrous cycle is 17 days long. Under most circumstances, ovulation occurs near the end of the estrus in the ewe, 25 to 30 hours after the beginning of estrus. In rare instances ovulation may occur even though the ewe is not in heat. This phenomenon occurs naturally during so-called "semiestrous" periods just before the sexual season (*4, 8*). In these semiestrous periods, anatomical changes characteristic of estrus occur in the ovaries, uterus, and vagina even though the desire for mating is absent. The semiestrous periods, usually only one in each animal, are synchronized with successive true estrous periods. Cole and Miller (*3*) produced a similar condition experimentally by injecting

[6] Italic numbers in parentheses refer to "Literature Cited" at the end of this bulletin.

gonadotropic hormone into ewes during the nonbreeding season, commonly known as anoestrus. That is, these ewes ovulated, but did not show estrus. If a second injection was given 17 days after the first, a considerable number came into heat, ovulated, and, if bred, became pregnant. It was deemed that such a procedure might prove to be a practical expedient in producing early spring lambs; but the work to date indicates that results have been too erratic for commercial usefulness. This hormone is obtained from the blood serum of pregnant mares at certain stages of pregnancy, and, when injected beneath the skin, stimulates the normal functioning of the ovary in female and the testicle in male animals. The work of this station does indicate that ovulation in the ewe depends upon the level of gonadotropin in the blood, as has been found to be true in laboratory experimental animals, particularly the white rat. Ovulation can be produced in almost 100 per cent of the ewes treated during anoestrum. The induction of estrus by this procedure, however, is inconsistent. As Cole and Miller (*4*) have shown, one can produce estrus easily and consistently by injecting another sex hormone, estrogen; but this hormone alone does not produce ovulation. The simultaneous production of ovulation and estrus by the combined administration of the two hormones, gonadotropin and estrogen, was not successful.

All (*4, 33, 39*) agree that ovulation, the release of the ovum from the ovary by rupture of the follicle in which it develops, occurs near the end of estrus, usually between the 25th and 30th hours after estrus begins. According to Cole and Miller (*4*) estrus usually lasts from 30 to 40 hours. The question naturally arises whether there is any particular time during estrus when mating is more likely to result in fertilization. In the work of Quinlan, Maré, and Roux (*40*) a lowered fertility apparently occurred when ewes were bred later than 30 hours after the beginning of estrus instead of earlier in estrus. This fact was taken to indicate only a short duration of viability of the liberated ovum. McKenzie and Phillips (*32*), on the contrary, found that 100 per cent of ewes conceived after the 14th hour of estrus, whereas only 66 per cent conceived when bred earlier in estrus. In both these studies the number of ewes in a group was too small to yield statistically significant information. The fact that estrus usually lasts only 30 to 40 hours would make it probable that the stage of estrus at which mating occurs is relatively unimportant in determining the percentage of fertile matings. Hartman (*15*) cites experiments with several species which show that the viability of ova in the oviduct decreases after the 12th hour. As ovulation usually occurs in the ewe between the 25th and 30th hours of estrus and, since the period lasts only 30 to 40 hours, the egg is likely to be viable from the time it is shed to the end of estrus in practically all instances. In this connection

we must also consider the viability of the sperm in the female genital tract: if a ewe is bred at the beginning of estrus, 25 hours or more will elapse before the egg is present for fertilization. Quinlan and co-workers (*40*) found live sperm in the reproductive tract of the ewe 48 hours after mating. Apparently, therefore, normal sperm will live until the egg is shed from the ovary.

Twinning.—The percentage lamb crop depends to a considerable extent upon the frequency of twinning. Twinning, in turn, largely depends upon the number of ova liberated from the ovary. When Hammond (*13*) killed and examined 80 pregnant ewes, he found 116 corpora lutea in the ovaries, 101 normal fetuses and 8 atrophied ones in the uteri; this left only 7 eggs to be accounted for. In adult pigs he found fertility to be dependent upon the percentage of fetuses developing normally, whereas in sheep, fertility depended largely upon the number of eggs shed at the heat period. As Kelley (*19*) has shown, the largest percentage of twins in Merino ewes is produced between four and ten years of age. The fertility of young sows is less than that of mature sows because fewer ova are shed (Hammond, *12*); and no doubt there is a similar relation between age and twinning in ewes. Jones and Rouse (*18*) concluded, from the literature covering a variety of species, that in animals generally the litter size increases along with the age of the female. This increase usually goes to a maximum, followed by a decline.

The relation of the ram to twinning has long been disputed. Twins are usually considered to be the result of two Graafian follicles rupturing at the estrous period, thereby releasing two ova. A single service deposits in the vagina an enormous number of spermatozoa, only one of which is required to fertilize each ovum. Therefore, as a rule, multiple ova must be present if multiple births are to occur. In one form of twinning, a single fertilized ovum divides into halves at the first cleavage stage; and from these two single cells two complete individuals are produced. These are monozygotic or identical twins, originating from a single zygote (fertilized egg).

Newman (*37*) states: "The majority of workers with human twins have had great difficulty in arriving at a clean-cut classification of their cases into monozygotic and dizygotic pairs. Finally it is now my settled conviction that there are two distinct categories of twins and that overlapping of the two groups is only a superficial one that can be resolved by careful study of even the most refractory cases."

In the human family it was thought that two fetuses in one membrane were monozygotic; two enclosed in separate membranes, dizygotic. Verschuer (*43*) showed that this is not always the case. All 32 of the monochorial pairs that he examined were one-egged. In 100 dichorial

pairs, only 76 were two-egged and 24 were one-egged. His explanation rests upon the fact that the ovum is fertilized in the oviduct and implanted in the horn of the uterus. The splitting occurs sometime before implanting, resulting in separate implants and in the development of two membranes. Verschuer was able to establish this in 16 out of 24 dichorial one-egged pairs.

Southwick (*42*) studying sex ratios and twin-producing kindreds, cites references indicating that only dizygotic twins are hereditary, the monozygotic pairs being spontaneous in all cases. Other references attempt to demonstrate a hereditary basis for both types of twinning. From his own studies in man, Southwick summarizes that an association exists between the hereditary factors for the production of identical and fraternal twinning.

In domestic animals it is even more difficult than in man to differentiate between the two classes of twins, and monozygotic cases appear to be relatively less frequent than in man. Conceivably, the factor responsible for such cases may be supplied by the sperm, and thus the male may be responsible.

Crew (*6*) states: "Selection is now being practiced by certain breeders to secure 100 per cent calf crop each year, and others are busily building up strains in which identical twins are the rule. In such an endeavor as this an animal breeding research department can be of service."

So, too, on theoretical grounds based on our knowledge of genetics, males may possibly transmit to their female offspring a tendency toward multiple ovulation. Lush (*22*), at the Kansas Experiment Station, studied inheritance of twinning in a herd of Holstein cattle in which 16 pairs of twins occurred among 181 births. Twelve pairs were dropped in four and a half years as compared with 4 pairs in the previous eight years. This increase, out of all proportion to the number of changes in the animals in the herd, resulted from the siring of numerous twins by a single bull. The twinning percentage in the Holstein herd was 8.84 per cent, or about five times as high as for other breeds in the Station herd, and many times higher than the average for dairy cattle. The tendency for twinning occurred more often in certain families. One out of every 5 cases bred to one sire produced twins. The cause was probably the bringing together in this animal of hereditary factors for twinning in the bull Hengerveld de Kol.

Davenport (*7*) gathered statistics on the influence of the male in producing human twins. In 355 labors occurring to mothers of twin-producing mothers 16 or 4.5 per cent were twins, while in 289 labors occurring to the mothers of twin-producing fathers 12 or 4.2 per cent were twins. Thus the frequency of twins in the fraternities of fathers

of twins was about equal to twins in the fraternities of mothers of twins, and four times greater than in the general population. In 30 families with identical twins the mothers came from fraternities where 13 per cent of 77 labors were twins, and the fathers from fraternities where in 38 labors 13 per cent were twins; and these were 12 times the frequency of twinning that occurs in the general population.

Despite this good evidence that the male may directly affect twinning through the influence of the sperm upon the formation of two zygotes from a single egg, there is very little evidence that monozygotic twins are common in sheep. As Clark (*2*) points out, if monozygotic twinning does occur, one should expect a departure from the ratio of 1 set of twin males, 2 sets of mixed twins, and 1 set of twin females, since identical twins must be of the same sex. Data collected by Clark (*2*), Chapman and Lush (*1*), and Johansson (*17*) do not depart from this expectancy; and thus one must conclude that monozygotic twins are rare in sheep. One must not infer, however, that the ram does not influence twinning: the male can presumably influence the number of ova shed by his daughters. Thus the preference of British flockmasters for breeding a ram that is one of twins rather than a single seems reasonable. The rhyme of Youatt, published in 1839, shows how old this idea is among practical sheepmen:

> Ewes yearly by lambing rich masters do make:
> The lambs of such twinners for breeders go take. *(44)*

Heredity and Fertility.—The fact that heredity influences fertility in sheep is attested by variations in fertility in different breeds, as shown by the data of Marshall and Potts (*25*). Ritzman and Davenport (*41*) give results of inbreeding on fertility in sheep. Mass production with matings that were not arranged particularly with reference to twinning traits caused the percentage lamb crop to remain practically unchanged from the original percentage down through four generations. There were 36 established family groups from 36 F_1 ewes for a study of fertility by the relative production of singles, twins, and triplets; but only 8 per cent of these families survived to the fourth generation. One of these families, A44, in three generations produced 1 set of triplets, 16 pairs of twins, and only 1 single lamb. One other family, A23, had 6 pairs of twins, and no singles in the second generation; 1 set of triplets, 2 pairs of twins, and 1 single in the third generation. In the fourth generation 3 of 7 ewes maintained high fertility.

Effect of Frequency of Breeding on the Male.—Another factor affecting fertilization is the frequency of breeding the male. Kirillow and Morosow (*20*) found bulls capable of maintaining a high sperm count, in one case up to the 24th ejaculation in 27 hours. According to Kusne-

zowa and her co-workers (*21*), one ram still ejaculated 100 million spermatozoa after 42 ejaculations over a 9-hour period. The first ejaculation contained 2.5 billions. McKenzie and Berliner (*34*) have similar information, although these authors did not test the fertilizing capacity of the semen after frequent ejaculations. Practical observations show that one ram is usually mated to 40 or 50 ewes under range conditions. In exceptional cases a ram may serve 100 ewes in one season. Under hand-breeding conditions, 200 to 300 ewes may be bred by one ram in a single breeding season. It is stated that older bucks tend to walk through the ewe band hunting for those in estrus and will breed each ewe only once. Yearling bucks tend to stay with a particular ewe found in heat and therefore breed fewer ewes during the season.

Implantation.—The successful implantation of the fertilized ovum and the maintenance of this attachment to the uterus throughout pregnancy both depend upon a functioning corpus luteum, or yellow body, in the ovary. The corpus luteum replaces the ovum and the follicular fluid when the ovum is shed. The corpus luteum secretes the hormone (progesterone) largely responsible for the attachment between the fetal and maternal membranes. In some species, particularly the human, the inadequate secretion of this hormone in certain individuals causes physiological abortion, which may be prevented by properly spaced injections of the hormone. Physiological abortion is rare in sheep and presumably in our other domestic animals. If there is insufficient progesterone very early in pregnancy, resorption of the fetus rather than abortion may occur. Cole and Saunders (*5*) have evidence that resorption does occur in the mare; and such resorption can be determined, for one can accurately diagnose early pregnancy in this species by the presence of gonadotropic hormone in the blood. In other domestic animal species, including sheep, in which this hormone does not appear in the blood, there is no accurate means of detecting early pregnancy, and it is difficult to obtain information on resorption. Data are presented later on the role which nutrition plays in the maintenance of pregnancy.

Parturition.—In the ewe, parturition, or the expulsion of the fetus from the womb, occurs 142 to 156 days after mating (Quinlan and Maré, *39*). According to these authors, working on Merino ewes in South Africa, most ewes lamb between the 146th and 152d day. The factors initiating the act of parturition are still unknown. The injection of gonadotropic hormone will prolong gestation in some species; and, conversely, pitocin, a hormone from the posterior lobe of the pituitary gland, is sometimes efficacious in precipitating parturition. There is, however, no evidence that these hormones are concerned in determining the duration of pregnancy under normal conditions.

Care of the Newborn.—The final stage in determining percentage lamb crop is raising the lamb after birth. Mortality in young lambs is a serious problem. Several factors enter into the success of rearing lambs: the strength of the lamb at birth; the amount of milk secreted by the ewe; the owning of the lamb by the mother, or maternal instinct; environmental conditions, such as temperature and humidity; and, finally, management practices.

Management involves many factors. Supervision 24 hours a day during the period is said to be the price that the sheepman pays for successful lambing. His presence is constantly needed to assist ewes having difficulty in lambing, to prevent the fetal membranes from suffocating the lamb, to help weak lambs suckle, and to place ewes with the newborn lambs in small pens or "jails" during the first 24 hours to facilitate acceptance of the lamb, a procedure more important with twins.

When feed is poor and ewes become too thin, they have little or no milk flow at lambing and will disown their lambs, being commonly termed poor mothers. This condition, usually a nutritional problem, can be remedied by supplemental feeding; otherwise, losses may be severe.

The desirability of using lambing sheds is controversial. The problem has been discussed, with illustrations, by Miller and Fermery (*35*). Sheds are necessary for protection in storms; but they tend to increase losses from infectious agents, causing dysentery, foot rot, sore mouth, navel ill, and the like. Since navel infection is widespread whether sheds are present or not, newborn lambs should always have tincture of iodine applied to the navel. Where lambing sheds are used, bedding is needed to keep the surface dry and clean. Fresh clean water should be available to the animals at all times.

Sheepmen commonly separate ewes heavy in lamb from the main band at biweekly intervals in order to give them particular attention. The selection is made according to udder development, and the process is termed "bagging out ewes." It is essential when lambing facilities are limited.

To supply a ewe that has lost her lamb but is still giving milk (a "wet" ewe) with a twin lamb from another mother is good practice. This is termed "grafting lambs" and is best carried out by skinning the dead lamb and placing the pelt on the lamb to be transferred. This lamb and its foster mother are then penned together for a day or two until the ewe owns the new lamb. It is desirable to have each ewe raise a lamb for the percentage of lambs saved may be the difference between profit and loss.

THE EFFECT OF NUTRITION ON REPRODUCTION

Now that we have discussed the various reproductive processes, let us consider the literature describing the effect of nutrition upon reproduction. On this subject few critical studies have been made.

Results Obtained by Other Workers.—Sheepmen have long considered it desirable to increase the feed shortly before breeding season in order that the ewes may gain in weight during the breeding period. The increase in feed may be either luxuriant pasture or the addition of grain to the ration. This practice is known as flushing. Marshall and Potts (*25*) who flushed ewes with grain and with pasture, found no difference in the results of the two procedures. The average number of lambs dropped per 100 ewes was 128.7 for the unflushed and 147.4 for the flushed group. There was a total of 143 ewes in the unflushed groups, 209 in the other. These results agree with the commonly accepted view. Clark (*2*) studied the effect of flushing on the number of eggs released from the ovary. He found that the number was increased by flushing if the ewes were thin, but not if they were in high condition before the flushing. In thin, unflushed ewes an average of 1.0 ovum apiece was released during a given estrus; in thin, flushed ewes, an average of 1.4. For the animals in high condition the averages were 1.5 for the flushed, 1.7 for the unflushed. Clark states: "From these results it would seem that the broad generalization that a rising condition, indicated by an increase in body weight, results in an increased ovulation rate, must be tempered by giving due emphasis to the condition of the ewes prior to the flushing period." The results of various investigators seem uniformly to indicate that flushing is beneficial if the ewes are thin. No one has yet ascertained whether it is better to keep the ewes relatively thin and then allow them to gain rapidly during the breeding season, or to keep them uniformly in good condition. Some sheepmen believe that change of feed during the breeding season, for better or worse, is to be avoided if possible.

Heape (*16*) of Cambridge University found in a survey that English flockmasters were uniformly agreed that excessively fat rams or ewes were poorer breeders than those in vigorous, healthy condition. Though practically all breeders would probably agree, we do not know of a single controlled experiment that would tell us just what degree of fatness becomes detrimental or how long an animal must be fat before its reproductive capacity is impaired.

Heape, surveying records from 306 flockmasters, found that fertility varied from 203.8 to 59.04 per cent, with an average fertility from 107,603 ewes of 120.4 per cent. The highest lambing percentages were found in small flocks. According to an analysis of the 120.4 per cent

lamb crop, 30 ewes produced twins, resulting in 60 lambs, whereas 60 ewes produced singles. Thus 90 ewes produced 120 lambs and 3 ewes died, while 7 were barren or aborted; thus 100 ewes produced 120 lambs. Such a percentage lamb crop is figured on the number of lambs born at term compared with the number of ewes at breeding time.

Lamb-Crop Data from California Producers.—In this state the percentage of dry or barren ewes is sometimes excessive, particularly in the flocks bred for early lamb production. The condition is associated with

TABLE 1

Data on Lambing Percentage and Breeding Practices on Sheep Farms in Three Sections of California during 1935, 1936, and 1938

Data requested in questionnaire	Sacramento Valley	San Joaquin Valley	Mendocino County
Number of replies	54	22	12
Total number of ewes	103,668	65,159	8,357
Per cent yearling ewes	22.8	13.3	14.0
Number of ewes at marking time	97,277	62,042	7,935
Number of lambs marked	91,792	60,979	6,313
Per cent lamb crop*	94.4	98.3	79.6
Per cent dry ewes†	9.6	6.5	11.9
Number of ewes per ram	41.8	64.4	36.6
Breeding period, days	88.9	82.0	76.3
Per cent of growers conditioning rams	53.7	50.0	66.6
Per cent of growers using ram-relay system	45.5	18.1	33.3

* Based on the number of lambs marked in relation to the number of ewes on hand at marking time.
† Includes a small percentage of ewes that lost their lambs and failed to raise twin lambs from other ewes.

the summer breeding season. In these flocks it varies from 5 to 20 per cent, whereas under the fall breeding season of northern California it is rarely over 6 to 8 per cent.

To obtain information on this subject a questionnaire was circulated in 1935, 1936, and 1938 among a number of progressive sheepmen in the early-lambing districts of California and in Mendocino County, which is outside this district.

Table 1 summarizes the data thus obtained, though its limitations are recognized because of difficulties in getting accurate figures.

The prevailing feed at breeding time on most of the farms supplying figures was grain stubble in the valleys and dry range feed in Mendocino County. Breeding in the latter area is done in early fall, when feed conditions are particularly rigorous. Practically all of the sheepmen replying recognized the desirability of giving green feed during breeding along with the dry feed.

Experimental Procedure.—In 1927, 125 high-grade yearling Rambouillet ewes were purchased, and the following year 80 Romney-Rambouillet cross-bred ewes were added. These ewes were used for experi-

mental purposes (*36*), one object being to study the effect of nutrition on the size of lamb crop.

The Rambouillet ewes came from a long-established flock in which purebred rams had been used for twenty-five years. The flock, numbering 6,000 to 8,000 ewes, had ranged throughout the year on the west side of the San Joaquin Valley. It had a lambing record of 80 to 100 per cent. The Romney-Rambouillet ewes came from a flock of about 2,000 head, which had ranged throughout the year in the Sacramento Valley. The average lamb crop in this flock was about 95 per cent. This lamb-crop percentage was figured on the number of lambs marked at about 2 weeks of age per 100 ewes bred. When these ewes came to the University Farm, where feed conditions were better most of the year, the average lamb production (figured on the same basis) rose to 125.5 per cent in the Rambouillet ewes, 127.6 per cent in the Romney-Rambouillets, for the six and four years, respectively, that the ewes were in the breeding experiment. For the three years in which the ewes were in their prime, several lots produced 160 to 165 per cent lamb crop.

These observations suggested the advisability of ascertaining whether any particular dietary essential is necessary for high fertility or whether the problem is merely one of total energy intake. Consequently an investigation was carried on over a seven-year period from 1932 to 1939. The deficiencies in the diet were mainly those of vitamin A, protein, and phosphorus. These deficiencies and the periods of time over which they continued were made to simulate natural range conditions.

Under range conditions in California the usual lamb crop is 80 to 90 per cent, figured on the number of lambs marked or docked to the number of ewes bred. In some of the experiments reported rigid control lots were not maintained; where not given, results were evaluated against the average lamb-crop production of the University Farm flock of purebred sheep covering a period of seven years comparable with the duration of these tests. This flock was maintained under optimum conditions in regard to nutritional requirements.

The earlier studies, carried on yearly from 1932 to 1935 inclusive have been published by Hart and Miller (*14*). A résumé is here presented.

Low Vitamin A during Breeding in Mature Ewes.—In 1932, 72 of the Romney-Rambouillet ewes previously mentioned, then five years old, were divided into three lots, to be fed diets as follows:

Lot 1. Low-vitamin-A basal diet of oat hay and grain mixture during breeding. The grain mixture consisted of 50 pounds of dried molasses beet pulp, 20 pounds of cottonseed meal, 20 pounds of rolled barley, and 10 pounds of wheat bran.

Lot 2. Low-vitamin-A basal diet plus cod-liver oil during breeding.

Lot 3. Control group, maintained on pasture according to usual farm practice.

During the breeding season, from August 6 to October 7, these animals in the respective lots made an average gain of 10.3, 17.1, and 23.4 pounds per head. Table 2 gives the lambing data.

The restricted diets of lots 1 and 2 affected the lamb crop adversely. Very low vitamin-A intake from May 14 to October 7 in lot 1 did not significantly lower the number of lambs in comparison with lot 2. The addition of 10 cubic centimeters of cod-liver oil daily to lot-2 animals during the breeding period had no significant effect on reproduction. It may have been a factor in the increased weight in these animals over those in lot 1.

TABLE 2

EFFECT OF VARIATIONS IN THE VITAMIN-A CONTENT OF THE RATION DURING BREEDING ON THE LAMBING RECORD OF EWES, SPRING OF 1933

Lot number and diet*	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb crop, per cent†
1. Basal ration, low vitamin A	23	19	3	0	1	25	108.7
2. Basal ration plus cod-liver oil	22	14	5	1	2	27	122.7
3. Control group, adequate diet	23	9	13	0	1	35	152.2

* One single lamb died shortly after birth in lot 1. In lot 2 the ewe with triplets died before parturition, 1 pair of twins and 1 single lamb were born dead, and 1 single died shortly after birth. In lot 3, 5 twin lambs, and 2 singles died soon after birth.

† Based on the number of lambs alive or dead in relation to the number of ewes bred.

Low Vitamin A and Low Protein during Breeding in Mature Ewes.—In 1933, 80 head of the Rambouillet ewes previously mentioned, then seven years old, were placed in dry lot on oat hay and grain mixture. They were divided into four lots on June 8 and fed as follows:

Lot 1. Low-vitamin-A diet.

Lot 2. Low-vitamin-A diet plus cod-liver oil during breeding.

Lot 3. Low-vitamin-A diet plus sudan-grass pasture during breeding.

Lot 4. Low-vitamin-A diet and low-protein diet plus cod-liver oil during breeding.

For the first three lots the roughage was a proprietary feed containing 60 pounds of cottonseed hulls, 25 pounds of cottonseed meal, and 15 pounds of cane molasses. The grain mixture consisted of 60 pounds of dried molasses beet pulp, 20 pounds of rolled barley, and 20 pounds of wheat bran.

Lot 4 received oat hay, and a grain mixture containing 75 pounds of dried molasses beet pulp and 25 pounds of barley. On July 7, lot 4 was

gradually changed from oat hay to cottonseed hulls, the protein thus being reduced from 8 to 5.5 per cent. The protein in hulls is only 20 per cent digestible. On August 1, all the lots began to receive cottonseed hulls. On that date, accordingly, the grain mixture for lots 1, 2, and 3 had to be changed to 50 pounds of dried molasses beet pulp, 30 pounds of cottonseed meal, 10 pounds of rolled barley, and 10 pounds of wheat bran.

After 3½ months on the deficient diet, 1 animal from lot 2 was killed, and the liver storage of vitamin A determined. It was found to

TABLE 3

Effect of Variations in the Vitamin-A and Protein Content of the Ration during Breeding on the Lambing Record of Ewes, Spring of 1934

Lot number and diet*	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb crop, per cent†
1. Basal ration, low vitamin A	14	5	6	2	1	23	164.3
2. Basal ration plus cod-liver oil	15	6	8	1	0	25	166.6
3. Sudan-grass pasture	14	3	9	0	2	21	150.0
4. Low vitamin A and protein plus cod-liver oil during breeding	14	6	5	1	2	19	135.7

* In lot 1, 2 lambs of 1 set of triplets were born dead and 1 single died shortly after birth. In lot 2, 2 of the triplets and 2 singles were born dead; 1 ewe with prolapsed vagina died and twin lambs were found in her uterus. In lot 3, 1 single lamb was born dead and 1 twin died shortly after birth. In lot 4, 1 single lamb was born dead.

† Based on the number of lambs alive or dead in relation to the number of ewes bred.

be 4,000 units per gram. Evidently, then, the high vitamin-A storage in these aged ewes was not being depleted very rapidly.

Eighteen ewes were then separated from the lots without breeding. They were maintained on the same low-vitamin-A diet as lot 1 until they were slaughtered. These animals were killed at intervals of about 2 months, and their livers tested for vitamin-A storage to determine the rate of depletion. Withdrawal of storage proved to be more rapid during the first year than during the second. The last of this group of ewes, just maintaining themselves without the drain of reproduction and lactation, were killed *in extremis* when over two years on the deficient diet. Night blindness did not appear until 22 to 23 months had elapsed.

The breeding of the remaining animals in the four groups covered the period from September 10 to October 12, after which they were taken off the restricted diets and turned out with the main farm flock. Ten cubic centimeters of cod-liver oil per ewe were supplied daily to lots 2 and 4 from September 2 to October 12. Table 3 gives the lambing data.

The results of this experiment, coupled with those of the previous year, showed that the animals were not being at all seriously depleted of vitamin A by the methods used. According to other studies (with

cattle) by Guilbert and Hart (*9, 10*), heat, ovulation, breeding, and implantation of fertilized ova are not interfered with until very late in vitamin-A deficiency. Gestation was affected, the result being the birth of dead or weak offspring.

The average weight changes of the ewes during the 10 days prior to breeding and the 32 days of the breeding period in the respective lots were + 2.6, — 0.4, + 7.6, and + 3.1 pounds. This, according to the general empirical requirements, was not enough gain to be considered true flushing. The percentage lamb crop in all lots was high, but the low-protein lot was 25 per cent lower than the average of the other three lots. Evidently, therefore, actual gain in weight is not necessary for a high-percentage lamb crop, provided the ewes are in sufficiently good condition and have enough body stores of the essential food substances.

It was concluded that the deficiency problems would have to be studied with young and growing ewes that had not had the opportunity to store large reserves of the needed essentials in their bodies.

Low Vitamin A during Breeding and Gestation in Ewe Lambs.—In 1934–35, ewe lambs were used; and, contrary to the previous procedure, the low-vitamin-A diet was maintained throughout the gestation period. A group of 61 lambs purchased June 21, 1934, was maintained on barley stubble until July 24. One lamb was carried in reserve and the others were divided into two lots to be fed as follows:

Lot 1. Low-vitamin-A diet: the cottonseed hulls and grain mixture used the previous year after the proprietary meal had been stopped.

Lot 2. Ladino-clover pasture.

When the extra lamb was killed on July 25, its liver contained 750 units of vitamin A per gram. One animal from each lot was killed on October 22; their livers contained 1,000 and 1,250 units respectively of vitamin A per gram.

The breeding season covered 36 days from October 26 to December 1, after which the lot-1 animals were kept on the same diet in dry lot and the lot-2 were handled according to the usual practice with the main farm flock. During this time lots 1 and 2 gained 5.3 and 7.8 pounds per ewe respectively.

On February 15 1 ewe from each lot was killed. The lot-1 animal had 250 units, and the lot-2 animal 3,300 units, of vitamin A per gram of liver tissue.

Lambing began on March 21, and in lot 1 2 animals showed definite and 1 showed partial night blindness on that date. On March 29 1 pregnant ewe in lot 1 with prolapsed uterus was killed, and her liver showed 25 units of vitamin A. On April 8, 9 of lot 1 were night-blind. They were placed in individual pens and fed definite amounts of caro-

tene, cod-liver oil, or dehydrated alfalfa meal in a study of minimum requirements of sheep for vitamin A by Guilbert, Miller, and Hughes (*11*). On April 20 the remaining 18 ewes in lot 1 were partially or completely night-blind. The last lamb from lot 1 was born on April 15; from lot 2 on April 24. Table 4 gives the lambing data.

At the close of this year's test all but 18 of these ewes were turned out to pasture as one group; this allowed them to restore any nutritional deficiencies, in preparation for the next year's experiment. The group

TABLE 4

EFFECT OF VITAMIN-A DEPLETION AND OF VARIATIONS IN THE VITAMIN-A CONTENT OF THE RATION DURING BREEDING AND GESTATION ON THE LAMBING RECORD OF EWE LAMBS, SPRING OF 1935

Lot number and diet*	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb crop, per cent†
1. Basal ration, low vitamin A	28	8	0	0	20	8	28.6
2. Ladino-clover pasture	27	13	0	0	14	13	48.1

* In lot 1, 7 lambs died shortly after birth. In lot 2, 1 lamb was born dead, and 1 died shortly after birth.
† Based on the number of lambs alive or dead in relation to the number of ewes bred.

of 18 ewes was continued on low-vitamin-A diet for the remainder of the season and were later used as lot 1 of the 1935–36 experiment.

The most interesting result of this year's experiment developed from continuing the ewes of lot 1 on a low-vitamin-A ration throughout gestation. It was found that even young growing ewe lambs which had had access to one season of green feed as nursing lambs stored sufficient vitamin A to enable them to remain on a low-vitamin-A diet from June 21, 1934, to March 21, 1935, a period of 9 months, before night blindness occurred. When breeding started on October 26, these ewes were not much lower in vitamin A than the lot-2 animals, as was determined by liver analysis of 1 animal from each group. The animals of lot 1 did not do well on the vitamin-A-deficient ration and did not have so high a percentage of pregnancies as those of lot 2. In lot 2, subjected to more favorable conditions than lot 1, the animals were more thrifty; this fact probably accounts for the larger lamb crop. Vitamin A cannot be considered the cause of the difference, as the data for the next breeding season clearly show. No ewe in either lot had twins or triplets. The very significant fact is that in lot 1 the lambs were born weak, and all but 1 died within 5 days, whereas in lot 2, 1 lamb was born dead, 1 died shortly after birth, and the remaining 11 were normal. Since ewe lambs have comparatively few estrous cycles during the breeding season, as shown by Cole and Miller (*4*), one may expect more or less difficulty in repro-

duction and a low-percentage lamb crop when breeding animals of this age.

As the evidence clearly shows, vitamin A is highly important in gestation. A high death rate of fetuses in the uterus and a high mortality of living offspring, which are usually born weak, may be expected if stores of vitamin A become too low in ewes toward the end of gestation.

Effect of Low Vitamin A during Breeding and Gestation; and of Low Protein and Phosphorus Compared with Low Protein and Adequate Phosphorus during Breeding.—In 1935–36, the work was virtually a continuation with the same group of ewes; one lot was further restricted in vitamin-A diet in order to have a group of animals still more severely depleted of this factor at breeding time.

On June 8 they were divided into three lots, each consisting of 18 head, fed as follows:

Lot 1. Low-vitamin-A diet: cottonseed hulls and grain mixture plus measured quantities of carotene, cod-liver oil, or dehydrated alfalfa meal to relieve night blindness. The grain mixture consisted of 66 pounds of dried molasses beet pulp, 30 pounds of cottonseed meal, and 4 pounds of decarbonized spent bone black.

Lot 2. Low-protein and low-phosphorus diet: cottonseed hulls and grain mixture plus cod-liver oil. The grain mixture consisted of 96 pounds of dried molasses beet pulp, 4 pounds of ground oystershell.

Lot 3. Low-protein diet: cottonseed hulls and grain mixture plus cod-liver oil. The grain mixture consisted of 96 pounds of dried molasses beet pulp, 4 pounds of decarbonized spent bone black.

Lot 1 included the 18 animals referred to above as on restricted vitamin-A diet, which had been fed vitamin A or its precursor in the study of minimum requirements by Guilbert, Miller, and Hughes (*11*). They were continued close to minimum levels throughout breeding and gestation. When their stores were exhausted and night blindness appeared, vitamin A was supplied in measured quantities as carotene, cod-liver oil, or dehydrated alfalfa meal of known carotene content.

Four ewes in lot 1 and 8 each in lots 2 and 3 had lambed the previous year. The total protein content in the diet of lot 1 was 12.8 per cent; that of lots 2 and 3 was 6.8 per cent. Vitamin A constituted about twice the minimum requirement in lots 2 and 3.

The animals in lot 2 soon began to chew the fences and appeared thin; this condition progressed, with the development of marked weakness. The breeding covered the 70 days from August 22 to October 31. During this time 3 of the ewes in lot 2 would have been too weak to support a ram if they had come into estrus. This condition was actually observed in one case.

At the end of the breeding season cod-liver-oil feeding to lots 2 and 3 was stopped, and both lots gradually changed to pasture with the main farm flock. One ewe in lot 1 died on January 3. She had twin lambs in the uterus, and the ewe's liver contained 3 units of vitamin A per gram. None of the lambs born to this group showed any trace of vitamin A in their livers. When the ewes were slaughtered, soon after lambing, their livers contained 8 to 80 units of vitamin A per gram. Table 5 gives the lambing data.

TABLE 5

Effect of Vitamin-A Depletion and of Variations in the Vitamin-A, Phosphorus, and Protein Content of the Ration on the Lambing Record of Ewes, Spring of 1936

Lot number and diet*	Total ewes	Ewes with singles	Ewes with twins†	Dry ewes	Total lambs	Lamb crop, per cent‡
1. Low vitamin A before and during breeding and gestation	17	9	2	6	13	76.4
2. Low protein, low phosphorus, with adequate vitamin A before and during breeding	18	7	2	9	11	61.1
3. Low protein, adequate phosphorus, with adequate vitamin A before and during breeding	18	12	3	3	18	100.0

* All of the lambs of lot 1 were born dead or died within 24 hours. In lot 2 a single lamb died at birth (posterior presentation requiring assistance). In lot 3 all lambs were alive and normal.

† No ewes had triplets.

‡ Based on the number of lambs alive or dead in relation to the number of ewes bred.

This was the first experiment in which any of the ewes had been so depleted of vitamin A as to show night blindness at breeding time. Half the ewes in lot 1 reached this state of depletion, and all had very low reserves. Except for the deficiency of vitamin A, the diet of lot 1 was better than that of lots 2 and 3, and the animals consumed more feed. Thus lot 1, despite symptoms of vitamin-A deficiency, still gained an average of 13.1 pounds from August 1 to October 31 and had a lambing record of 76.4 per cent. All of the lambs were dead at birth or were very weak and died within 24 hours.

Lot 2, on low phosphorus and protein, lost an average of 17.7 pounds from June 8 to August 1. From then until the end of the breeding period they approximately maintained their weight. This lot had a lambing record of 61.1 per cent. All the lambs were born healthy except 1 that died at birth. The appetite of lot 2 was noticeably poor, and depraved appetite was observed during breeding. The number of ewes lambing was 9 out of the 18, and 2 sets of twins were produced. Certainly the physical condition of the sheep in this lot was by far the worst of the three groups. There was, nevertheless, no significant lowering of blood

phosphorus by the regimen to which they were subjected. One may probably explain this fact by assuming that the vitamin D in the cod-liver oil drew phosphorus stores from the bones of the animals and so maintained blood-phosphorus levels at normal.

Lot 3 on low protein, but ample phosphorus and vitamin A, lost 0.3 pound per ewe from August 1 to October 31; and 15 of the 18 ewes lambed. As there were 3 sets of twins, a 100 per cent lamb crop was produced. All the lambs were alive and normal.

Judging from the data, low protein may not be so serious in its effect on fertility as low protein with low phosphorus. This hypothesis was not substantiated by the experiments begun in the fall of 1936. Animals depleted of vitamin A to the point of partial night blindness will still show estrus, will ovulate, breed, and become pregnant. For normal birth, however, ample vitamin A must be supplied during gestation.

The investigations were continued after the publication of the data collected from 1932–33 to 1935–36 and reviewed above.

Low Protein with Low Phosphorus and Low Protein with Adequate Phosphorus in Yearling and Two-Year-Old Ewes.—The 1936–37 experiment was a continuation of the 1935–36 program, using 34 of the same ewes, now two years old and in good condition.

An additional 26 grade yearling ewes, in fair condition but somewhat undersized, were purchased.

On June 1 the animals were segregated into two lots, each containing 17 two-year-olds and 13 yearling ewes, and were fed as follows:

Lot 1. Low protein and low phosphorus: cottonseed hulls; also a mixture of 96 pounds of plain dried beet pulp with 4 pounds of ground oystershell.

Lot 2. Low protein and adequate phosphorus: cottonseed hulls; also a mixture of 96 pounds of plain dried beet pulp with 4 pounds of decarbonized spent bone black.

The protein content was 5.5 per cent. The calcium-phosphorus ratio was 23 to 1 in lot 1, 3 to 1 in lot 2.

Cod-liver oil, used the previous year, was omitted because its vitamin-D content may have been a factor in maintaining blood phosphorus.

Because the palatability was low, the intake was below maintenance. After 2 months the ewes had lost weight, particularly the yearlings, and had very poor appetite. When dried molasses beet pulp was substituted for the plain pulp, appetite returned, and food consumption became more satisfactory.

The breeding period extended from September 1 to October 31. Two mutton-type rams, two years old, were rotated between the two lots.

They were placed with the ewes each evening and removed in the morning so that they could be fed a proper ration. Breeding progressed slowly, particularly among the yearlings, which were not eating well and looked unthrifty. Abnormal appetite was evidenced by chewing on

TABLE 6

Average Weight per Head of Ewes from June 1 to October 31, 1936, While on Low Protein with Low Phosphorus and on Low Protein with Adequate Phosphorus

Date	Lot 1 (low protein and low phosphorus)		Lot 2 (low protein and adequate phosphorus)	
	Mature ewes	Yearling ewes	Mature ewes	Yearling ewes
	pounds	*pounds*	*pounds*	*pounds*
June 1	124.5	80.6	124.7	75.3
July 31	123.2	73.7	122.9	71.0
August 31	120.5	74.2	120.1	69.7
October 31	108.3	69.0	112.5	63.0
Loss	16.2	11.6	12.2	12.3

TABLE 7

Blood-Phosphorus Levels in Ewes from June 1 to October 31, 1936, while on Low Protein with Low Phosphorus and on Low Protein with Adequate Phosphorus

Date	Average milligrams phosphorus per 100 cubic centimeters of blood serum			
	Lot 1 (low protein and low phosphorus)		Lot 2 (low protein and adequate phosphorus)	
	Mature ewes	Yearling ewes	Mature ewes	Yearling ewes
June 19	5.7	6.4	6.9	6.7
September 1	7.3	5.6	9.4	8.0
October 30	8.0	7.4	9.2	9.0

fence boards and eating dirt. Since appetite was falling off, the feed had to be reduced about 30 per cent during the last week in October. At the close of the breeding season all the animals were gradually changed to normal diet and placed with the main farm flock.

Table 6 gives the average weight data of the ewes; table 7 the blood-phosphorus determinations.

As will be observed, there was a gradual increase in the blood-phosphorus content of the animals in both lots. Since lot 1 was receiving very little phosphorus, this seeming contradiction can be explained only

by the fact that the animals were losing weight; emaciation makes possible the maintenance of blood levels on a low phosphorus intake.

Table 8 gives the lamb-crop data.

All the lambs were born to the mature ewes; not a single yearling ewe in either lot became pregnant. The lambing percentage was low, with no significant difference between the lots.

This year's studies showed that in yearling ewes low protein alone was as serious as low protein and low phosphorus. All the yearlings in both lots remained barren, because they were losing weight and

TABLE 8

EFFECT OF LOW PROTEIN WITH LOW PHOSPHORUS AND OF LOW PROTEIN WITH ADEQUATE PHOSPHORUS BEFORE AND DURING BREEDING, ON THE LAMBING RECORD OF EWES, SPRING OF 1937

Lot number and diet	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb crop, per cent*
1. Low protein with low phosphorus.............	30	6	7	0	17	20	66.6
2. Low protein with adequate phosphorus.......	30	10	2	0	18	14	46.6

* Based on the number of lambs alive or dead in relation to the number of ewes bred.

growth had not been completed. In the two-year-olds, 13 ewes lambed in lot 1 against 12 ewes in lot 2. This seeming difference from the results of the previous year is not great enough to be significant with this number of animals.

Low Protein with Low Phosphorus and Low Protein with Adequate Phosphorus in Two- and Three-Year-Old Ewes.—In 1937–38 it was desired to study further the effect of low phosphorus.

The ewes of the previous year were used again. The lambs of the previous year were sacrificed when a few days old, except 5 ewes whose lambs were weaned just before the experiment.

On May 30 the ewes were segregated into two lots of 30 head each, and on June 25 they were placed on the following diets:

Lot 1. Low protein and low phosphorus: dried molasses beet pulp 96 pounds, whey powder 10 pounds, and ground oystershell 4 pounds.

Lot 2. Low protein and adequate phosphorus: dried molasses beet pulp 96 pounds, whey powder 10 pounds, and steamed bone meal 4 pounds.

Cottonseed hulls were fed to both groups as roughage. Monthly weights were taken, and feed was supplied according to appetite. On the average, each ewe consumed daily about 1.3 pounds of the concentrate mixture and 1.2 pounds of cottonseed hulls.

On this basis lot 1 received a diet containing 8 per cent total protein, with a calcium-phosphorus ratio of 11.7 to 1. The lot-2 diet contained the same per cent of total protein, with a calcium-phosphorus ratio of 2.3 to 1.

Except for one fatality in lot 1 and two others in lot 2 the ewes remained through the summer in much better condition than in the previous year, apparently because of the whey powder in the feed.

TABLE 9

Average Weight of Ewes from June 2 to October 5, 1937, While on Low Protein with Low Phosphorus and on Low Protein with Adequate Phosphorus

Date	Average weight per head, in pounds			
	Lot 1 (low protein with low phosphorus)		Lot 2 (low protein with adequate phosphorus)	
	Three-year-old ewes	Two-year-old ewes	Three-year-old ewes	Two-year-old ewes
	pounds	*pounds*	*pounds*	*pounds*
June 2	131.8	98.1	130.8	95.1
July 1	125.2	93.6	124.5	91.4
August 1	125.2	92.2	127.4	91.1
September 1	123.4	93.1	122.9	94.0
October 5	119.9	90.6	120.4	88.9
Loss	11.9	7.5	10.4	6.2

The breeding period extended from September 1 to October 31, 2 Shropshire rams being alternated between the lots each night and separated in the day. The ewes began to breed uniformly. As the season progressed, however, the lot-1 animals slowed up in actual feed consumption; several appeared thin and unthrifty. With the close of the breeding season, both lots were gradually removed from the diets and placed with the main flock.

Blood samples were taken on September 3 and also on October 29 for phosphorus determination, with the following results:

	Milligrams per 100 cubic centimeters
Lot 1:	
September 3	7.64
October 29	7.89
Lot 2:	
September 3	8.80
October 29	9.86

The variations are not significant, and all were in the normal range of blood-phosphorus content.

Table 9 gives the weights of the ewes; table 10 the lamb-crop data.

The lambing percentage was calculated on the number of ewes at breeding time, which included the 3 that died.

As was to be expected from the blood-phosphorus level, the feeding regimen to which the two lots were subjected had no significant effect on the percentage lamb crop. The ewes were one year older during this experiment. They had been on normal diet with the farm flock for 7 months. Their good condition, coupled with high nutritive value of the whey, prevented interference with their reproductive functions over the period of 4 to 5 months despite loss in weight.

TABLE 10

EFFECT OF LOW PROTEIN WITH LOW PHOSPHORUS AND OF LOW PROTEIN WITH ADEQUATE PHOSPHORUS, BEFORE AND DURING BREEDING, ON THE LAMBING RECORD OF EWES, SPRING OF 1938

Lot number and diet	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Ewes dry	Total lambs	Lamb crop, per cent*
1. Low protein with low phosphorus	29	22	5	0	2	32	106.6
2. Low protein with adequate phosphorus	28	20	7	0	1	34	113.3

* Based on the number of lambs alive or dead in relation to the number of ewes bred.

Relation of Factors in the Vitamin-B Complex to Fertility.—The favorable effects of feeding the whey powder the previous year indicated the advisability of considering factors in the B complex in addition to the high-quality-protein content. At this time the synthesis of B factors in the rumen was being studied by McElroy and Goss (*26, 28, 29, 30, 31*) and by McElroy and Jukes (*27*).

Forty-eight of the ewes from the previous year were divided into four lots of 12 each on July 13, 1938. They were all placed on a basal concentrate ration consisting of 88 pounds of dried molasses beet pulp, 10 pounds of washed casein, 1 pound of steamed bone meal, and 1 pound of salt.

Cottonseed hulls were again fed to all lots as roughage. On August 8 the controlled feeding began as follows:

Lot 1. Basal ration.

Lot 2. Basal ration plus riboflavin as 1 per cent of the diet.

Lot 3. Basal ration plus riboflavin as 1 per cent of the diet and nicotinic acid 0.3 milligrams per kilo of body weight per day.

Lot 4. Basal ration plus riboflavin and nicotinic acid (same as lot 3) plus rice-bran filtrate factor 3 per cent of the diet.

The nicotinic acid, in solution, was given orally twice a week. The filtrate factor was dissolved in water and sprinkled on the feed twice daily.

Cod-liver oil was supplied to all lots at the rate of 10 cubic centimeters per head twice a week, sprinkled on the feed to insure an ample supply of vitamins A and D. A block of salt was placed in each corral in addition to that in the basal ration. The casein was washed to remove factors in

TABLE 11

AVERAGE WEIGHT PER HEAD OF EWES FROM JULY 13, 1938 TO MARCH 4, 1939, RECEIVING BASAL RATION ONLY OR BASAL RATION PLUS FACTORS IN THE VITAMIN-B COMPLEX

Date	Lot 1 (basal ration)	Lot 2 (same as 1 plus riboflavin)	Lot 3 (same as 2 plus nicotinic acid)	Lot 4 (same as 3 plus filtrate factor)
	pounds	*pounds*	*pounds*	*pounds*
July 13, 1938	128.0	121.4	124.8	125.6
August 18, 1938	134.2	128.0	127.4	134.5
September 17, 1938	137.8	133.6	133.0	137.9
October 19, 1938	148.4	146.2	141.1	139.2
November 19, 1938	136.2	135.1	131.2	136.9
December 15, 1938	144.8	149.4	145.5	146.0
March 4, 1939	153.2	149.0	146.9	154.3
Gain	25.2	27.6	22.1	28.7

TABLE 12

EFFECT OF BASAL RATION AND BASAL RATION PLUS FACTORS IN THE VITAMIN-B COMPLEX, BEFORE AND DURING BREEDING, ON THE LAMBING RECORD OF EWES, SPRING OF 1939

Lot number and diet	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb crop, per cent*
1. Basal ration	12	7	4	1	0	18	150.0
2. Same as 1 plus riboflavin	11	4	6	0	1	16	145.4
3. Same as 2 plus nicotinic acid	12	3	6	0	3	15	125.0
4. Same as 3 plus filtrate factor	12	4	7	1	0	21	175.0

* Based on the number of lambs alive or dead in relation to the number of ewes bred.

the vitamin-B complex. The basal ration which all groups received contained ample phosphorus and 12 per cent protein.

Under the feeding regimen all lots increased in weight. Four mutton-type rams were alternated among the four lots during the breeding season, which began on November 25, 3 months after the ewes had been placed on the diet. The rams were removed December 15, and all lots were gradually changed from the special diets and turned out with the main farm flock.

Lambing began March 27; and by April 15 all the ewes had lambed except 2 in lot 1, which lambed on April 20 and May 5, respectively.

Table 11 gives the weights of the ewes; table 12 the lamb-crop data. One ewe in lot 2 died before breeding began and was not counted. Three other ewes, which died after the breeding period, are included. One of these in lot 2 had twins in her uterus, whereas the other 2 in lot 3 were not pregnant.

The factors in the vitamin-B complex had no effect on the results. The experiment brings out strongly the evidence that the basal ration adequate in high-quality protein and in phosphorus caused all the lots to make relatively large gains, to breed over a short period, and to produce a high percentage of lambs.

FERTILITY OF THE FARM FLOCK IN THE YEARS COVERED BY THESE EXPERIMENTS

The experiments reported herein extended over the years 1933 to 1939. The University Farm sheep exclusive of the experimental animals during these years consisted of small purebred flocks of Rambouillets, Hampshires, Shropshires, Southdowns, and Romneys. The combined number of ewes of all ages for the five breeds varied in the different years from 89 to 131. The lambing percentages of these ewes for the respective years are given in table 13 to represent the normal fertility of the stock that occurred under conditions of presumably adequate nutrition as occurred in ordinary farm practice.

In the seven years there was a total of 797 ewes producing 991 lambs or an over-all crop for the seven years of 124.1 per cent. Results in the experiments may be compared with these as being representative of sheep kept under optimum conditions of care and feeding.

DISCUSSION

During the seven years of the experiments there were in all 434 sheep in the various lots, with 238 different animals. The number of animals per lot varied from 12 to 30 head.

In 1932 the control lot maintained on pasture according to usual farm practice made good gains during the breeding season, 23.4 pounds per head, and produced a high-percentage lamb crop. The same results were secured with all the lots in 1938. These ewes were on an adequate diet except for the vitamin-B-complex factors and made a favorable gain in weight during breeding.

In 1933 there were four lots. Lot 2, receiving a high intake of basal diet plus cod-liver oil, lost the insignificant average of 0.4 pound during the 32-day breeding season and 10 days prior thereto. These animals

had the second-highest percentage lamb crop (166.6) of any group during the seven years.

Evidently, therefore, gain in weight during the breeding season is not essential for high fecundity in ewes that are in good condition. This does not mean that thin ewes fed well during the breeding season may not produce more lambs than if they were left on poor feed. Under these conditions flushing is worth while even though it is not always essential to a high percentage of lambs.

TABLE 13

Lambing Percentages of Ewes in Farm Flock Other than the Experimental Ewes

Year	Number of ewes	Number of lambs	Lamb crop, per cent*
1933	97	122	125.7
1934	106	131	123.6
1935	130	157	120.7
1936	117	138	117.9
1937	131	177	135.1
1938	127	152	119.7
1939	89	114	128.1
Total and average	797	991	124.1

* Based on the number of lambs docked in relation to the number of ewes bred.

As the data show, a low intake of vitamin A requires many months to deplete breeding ewes of their vitamin-A storage. An advanced stage of depletion is necessary before estrus, ovulation, breeding, fertilization, and implantation are interfered with. Gestation, on the other hand, may become markedly affected, with the death of the fetus *in utero* or the birth of weak lambs that may die during the first few days of life.

Somewhat conflicting data were obtained on low protein alone and on low protein and low phosphorus. In 1933 low protein alone in lot 4 resulted in weight maintenance and a satisfactory lambing percentage although lower than the other three groups (table 3).

In 1935 low protein alone in lot 3 also resulted in weight maintenance and a reasonable lamb crop (100 per cent) compared to a serious loss of body weight and a very unsatisfactory lamb crop (61.1 per cent) in lot 2 on low protein and low phosphorus.

In 1936 and 1937 both low protein and low phosphorus and low protein alone caused about the same weight losses. The lamb crop varied markedly in the two years, being satisfactory in 1937 and low in 1936. The low lamb crop in 1936 was largely due to the fact that none of the yearling ewes in either lot lambed. This is further evidence of the sever-

ity of a restricted diet with immature animals. In 1937 all ewes had reached maturity and furthermore dried whey was added to the ration, which greatly improved the biological value of the protein.

In 1938, with adequate protein of high quality in casein and adequate phosphorus, high fertility was obtained as well as high weight gains in the ewes. This year's diets dealt with the vitamin-B-complex factors.

Experiments with high protein and low phosphorus were not included because this condition does not occur on the range. Under natural conditions on dry feed the protein may be lower than the levels maintained in this work. In average rations protein and phosphorus are closely associated, and low protein is usually accompanied by low phosphorus. The data present evidence that this condition should be avoided.

The studies of McElroy and Goss (*26*) at this station established that all the factors in the vitamin-B complex are produced in the rumen and that deficiency does not occur in sheep and cattle from the diet's being low in the various factors. The 1938 trial in the experiments reported here also showed this to be the case.

CONCLUSIONS

Vitamin A is a dietary factor the deficiency of which will affect normal reproduction in sheep. Normal body stores are depleted slowly. Absence of sufficient quantities in the feed over too long a period, as may occur, in dry years, can become a serious factor in lamb mortality.

Low protein and low phosphorus in the feeding regimen of breeding ewes must be avoided if condition of the ewes is to be maintained and high-percentage lamb crop secured.

The relative importance of these two dietary essentials could not be ascertained from the data. Stores of phosphorus are depleted slowly, whereas protein deficiency manifests itself in a shorter time.

The experiments show the importance of nutrition during breeding and gestation in the financial stability of a sheep-producing enterprise.

With protein supplements on dry range feed and with adequate intake, specific deficiencies are to be particularly reckoned with only under unusual conditions.

LITERATURE CITED

1. Chapman, A. B., and J. L. Lush.
   1932. Twinning, sex ratios and genetic variability in birth weight in sheep. Jour. Hered. **23**:473–78.

2. Clark, R. T.
   1934. Studies on the physiology of reproduction in the sheep. I. The ovulation rate of the ewe as affected by the plane of nutrition. Anat. Rec. **60**:125–34.

3. Cole, H. H., and R. F. Miller.
   1933. Artificial induction of ovulation and oestrum in the ewe during anoestrum. Amer. Jour. Physiol. **104**:165–71.

4. Cole, H. H., and R. F. Miller.
   1935. Changes in the reproductive organs of the ewe with some data bearing on their control. Amer. Jour. Anat. **57**:39–97.

5. Cole, H. H., and F. J. Saunders.
   1935. The concentration of gonad-stimulating hormone in blood serum and of oestrin in the urine throughout pregnancy in the mare. Endocrinology **19**:199–208.

6. Crew, F. A. E.
   1927. The organization and function of an animal breeding research department. V. Internationalen Kongresses für Vererbungswissenschaft. Verhandl. p. 169–82. Berlin.

7. Davenport, C. B.
   1920. Influence of the male in the production of human twins. Amer. Nat. **54**:122–29.

8. Grant, R.
   1934. Studies on the physiology of reproduction in the ewe. Part 1. The symptoms, periodicity and duration of oestrus. Part 2. Changes in the vagina and cervix. Part 3. Gross changes in the ovary. Roy. Soc. Edinb. Trans. **58**:1–47.

9. Guilbert, H. R., and G. H. Hart.
   1934. Storage of vitamin A in cattle. Jour. Nutr. **8**:25–44.

10. Guilbert, H. R., and G. H. Hart.
    1935. Minimum vitamin A requirements with particular reference to cattle. Jour. Nutr. **10**:409–27.

11. Guilbert, H. R., R. F. Miller, and E. H. Hughes.
    1937. The minimum vitamin A and carotene requirement of cattle, sheep, and swine. Jour. Nutr. **13**:543–64.

12. Hammond, John.
    1914. On some factors controlling fertility in domestic animals. Jour. Agr. Sci. [England] **6**:263–77.

13. Hammond, John.
    1921. Further observations on the factors controlling fertility and foetal atrophy. Jour. Agr. Sci. [England] **11**:337–66.

14. Hart, G. H., and R. F. Miller.
    1937. Relation of certain dietary essentials to fertility in sheep. Jour. Agr. Res. **55**:47–58.

15. HARTMAN, CARL.
1939. Ovulation, fertilization, and the transport and viability of eggs and spermatozoa. *In:* Allen's sex and internal secretions. 2d ed. 655 p. The Williams and Wilkins Co., Baltimore, Md.

16. HEAPE, W.
1899. Abortion, barrenness and fertility in sheep. Roy. Agr. Soc. England Jour. **10**:217–48.

17. JOHANSSON, I.
1932. Multiple births in sheep. Amer. Soc. Anim. Prod. Proc. 285–91.

18. JONES, S. V. H., and J. E. ROUSE.
1920. The relation of age of dam to observed fecundity in domestic animals. Jour. Dairy Sci. **3**:260–90.

19. KELLEY, R. B.
1939. Female aspects of relative fertility in sheep. Austral. Vet. Jour. **15**:184–98.

20. KIRILLOW, W. W., and W. MOROSOW.
1933. The influence of sexual activity of bulls on their reproductive ability. Problems in Animal Husbandry **5**:90.

21. KUSNEZOWA, N., W. MILOWANOW, O. NEWMANN, W. NAZAJEW, and P. SKATKIN.
1932. The artificial insemination of cattle and sheep. 528 p. Inst. Anim. Husb., Selkolschozghiz. U.S.S.R.

22. LUSH, R. H.
1925. Inheritance of twinning in a herd of Holstein cattle. Jour. Hered. **16**:273–79.

23. MARSHALL, F. H. A.
1910. The physiology of reproduction. 706 p. Longmans, Green and Co., London.

24. MARSHALL, F. H. A.
1936. Sexual periodicity and the causes which determine it. Roy. Soc. London Phil. Trans., Ser. B. **226**:423–56.

25. MARSHALL, F. R., and C. G. POTTS.
1921. Flushing and other means of increasing lamb yields. U.S. Dept. Agr. Dept. Bul. **996**:1–14.

26. MCELROY, L. W., and H. GOSS.
1939. Report on four members of the vitamin B complex synthesized in the rumen of sheep. Jour. Biol. Chem. **130**:437–38.

27. MCELROY, L. W., and T. H. JUKES.
1940. Formation of the anti egg-white injury factor (Biotin) in the rumen of the cow. Soc. Exp. Biol. and Med. Proc. **45**:296–97.

28. MCELROY, L. W., and H. GOSS.
1940. A quantitative study of vitamins in the rumen contents of sheep and cows fed vitamin low diets. I. Riboflavin and vitamin K. Jour. Nutr. **20**:527–40.

29. MCELROY, L. W., and H. GOSS.
1940. A quantitative study of vitamins in the rumen contents of sheep and cows fed vitamin low diets. II. Vitamin B_6 (Pyridoxine). Jour. Nutr. **20**:541–50.

30. MCELROY, L. W., and H. GOSS.
1941. A quantitative study of vitamins in the rumen contents of sheep and cows fed vitamin low diets. III. Thiamin. Jour. Nutr. **21**:163–73.

31. McElroy, L. W., and H. Goss.
1941. A quantitative study of vitamins in the rumen contents of sheep and cows fed vitamin low diets. IV. Pantothenic acid. Jour. Nutr. **21**:405–9.

32. McKenzie, F. F., and R. W. Phillips.
1930. Some observations on the estrual cycle in the sheep. Amer. Soc. Anim. Prod. Proc. 23d Ann. Meeting. p. 138–43.

33. McKenzie, F. F., E. Allen, Mary J. Guthrie, V. Warbritton, C. E. Terrill, L. E. Casida, L. J. Nahm, and J. W. Kennedy.
1933. Reproduction in the ewe. A preliminary report. Amer. Soc. Anim. Prod. Proc. 23d Ann. Meeting. p. 278–81.

34. McKenzie, F. F., and N. Berliner.
1937. The reproductive capacity of rams. Missouri Univ. Res. Bul. **265**:1–143.

35. Miller, R. F., and G. E. Fermery.
1917. Lambing sheds. California Agr. Exp. Sta. Cir. **188**:1–16.

36. Miller, R. F.
1935. Crossbreeding investigations in the production of California spring lambs. California Agr. Exp. Sta. Bul. **598**:1–32.

37. Newman, H. H.
1931. Differences between conjoined twins in relation to a general theory of twinning. Jour. Hered. **22**:201–15.

38. Nichols, J. R.
1927. Meteorological factors affecting fertility in the sheep. Ztschr. f. Induktive Abstam. u. Vererbungslehre **43**:313–39.

39. Quinlan, J., and G. S. Maré.
1931. The physiological changes in the ovary of the Merino sheep in South Africa and their practical application in breeding. Union So. Africa Dept. Agr. 17th Rpt. Dir. Vet. Serv. and Anim. Indus. p. 663–703. Onderstepoort, Pretoria.

40. Quinlan, J., G. S. Maré, and L. L. Roux.
1932. The vitality of the spermatozoon in the genital tract of the Merino ewe, with special reference to its practical application in breeding. Union So. Africa Dept. Agr. 18th Rpt. Dir. Vet. Serv. and Anim. Indus. p. 831–70. Onderstepoort, Pretoria.

41. Ritzman, E. G., and C. B. Davenport.
1931. Some results of inbreeding on fecundity and on growth in sheep. New Hampshire Agr. Exp. Sta. Tech. Bul. **47**:1–27.

42. Southwick, W. E.
1939. Sex ratios and twin producing kindreds. Amer. Nat. **73**:44–65.

43. Verschuer, F. von.
1939. Twin research from the time of Francis Galton to the present day. Roy. Soc. London Proc., Ser. B. **128**:62–81.

44. Wentworth, E. N., and J. B. Sweet.
1917. Inheritance of fertility in Southdown sheep. Amer. Nat. **51**:662–82.

45. White, R. G., and J. A. F. Roberts.
1927. Fertility and sex-ratio in Welsh mountain sheep. Welsh Jour. Agr. **3**:70–79.

12*m*-10,'42 (2240)